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REVIEW ARTICLE

Per- and polyfluoroalkyl substances in human breast milk and current analytical methods

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Abstract
Per- and polyfluoroalkyl substances (PFASs) have since become a major health concern as they have been reportedly found in human tissues, blood and breast milk. The main aim of the study was to review the current data on PFASs in human breast milk, including the challenges of analysis as well as the possible modes of transfer from maternal blood. In this paper, previously published data on the concentrations of PFASs in human breast milk from around the world were reviewed and summarised. Eligible studies with reference lists published before 1 June 2017 were included by searching several databases (including Scopus, ScienceOpen and SciFinder). From this search, studies with the number of participants in each study ranging from 2 to 1237 were identified. The review indicated that based on the structural profiles and concentration levels, there was variation in the geographical distribution of these compounds in breast milk. Although there are no recorded investigations on the modes of transfer from maternal blood to breast milk, literature suggests that the PFASs tend to be transferred through binding to various proteins. The review also examined the different sample preparation and analytical methods employed to measure the concentrations of PFASs in human breast milk. This showed that solid phase extraction was the most common extraction method. After extraction, liquid chromatography coupled with tandem mass spectrometry was the most common analysis method. Since several of these methods were initially dedicated to monitoring PFASs in food and water, they demonstrate some limitations with regard to specificity and sensitivity to human fluids. Additionally, there are currently no published records of certified reference materials and/or proficiency scheme devoted to standardising PFAS concentrations in breast milk.

Keywords Perfluoroalkyl substances · Perfluorooctanoic acid · Perfluorooctane sulphonate · Biomonitoring · Human breast milk

Introduction

Per- and polyfluoroalkyl compounds (PFASs) belong to a group of fluorinated substances manufactured in the 1950s for application in several commercial and industrial applications (Kissa 2001). PFASs can be defined as artificial organic compounds, having carbon chains of different lengths where their hydrogen atoms are completely (perfluorinated) or partially (polyfluorinated) replaced by fluorine atoms, except for those hydrogen atoms whose replacement would alter the nature of the functional groups, forming a sturdier carbon–fluorine (C–F) bond (Alexander et al. 2003). The high electronegativity of fluorine atoms strongly attracts shared electrons resulting in a compound with high chemical and thermal stability. The C–F bond is considered one of the strongest in nature, thereby making the synthesised product highly resistant to biological degradation (Mabury 2005). In addition, under suitable conditions, polyfluorinated compounds can be biotically or abiotically transformed into perfluorinated compounds in the environment (Buck et al. 2011).

PFASs are manufactured through two key processes, namely telomerization and electrochemical fluorination (ECF) (Fujii et al. 2007; Beesoon et al. 2011). The two processes yield two distinct types of products; whereas ECF produces short-chain PFASs and perfluorinated sulphonates with branched isomers, telomerization produces even numbered carbon chains, particularly perfluorocarboxylates (Fujii et al. 2007). By definition, short-chain PFASs are perfluoroalkyl carboxylic acids with seven carbons or less and, perfluoroalkane sulphonates with

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five carbons and less (Buck et al. 2011; Xiao et al. 2018). Moreover, it has been noted that the same PFAS produced by either process can be analytically distinguished, for instance, telomerised perfluorooctanoic acid (PFOA) is a linear isomer (Kissa 1994; Beesoon et al. 2011) and ECF PFOA is a combination of both branched and linear isomers (Loveless et al. 2006; Beesoon et al. 2011).

The unique properties of PFASs (Table 1), which enhance their ability to repel water, fat and dust as well as their high resistance to aggressive chemicals, make them popular among modern manufacturers (Kärrman et al. 2007). The table below shows some physico-chemical properties of perfluorooctanoic acid (PFOA), perfluorooctane sulphonate (PFOS), perfluorononanoic acid (PFNA), perfluorohexane sulphonic acid (PFHxS) and perfluorodecanoic acid (PFDA); which are some of the most frequently detected PFASs in human breast milk.

The physicochemical properties of PFASs are generally not well documented in literature and there remain inconsistencies among the available studies. In addition, much of the available data has either been predicted or calculated rather than derived experimentally and this is due to the limited number of pure PFASs available for such study (Ding and Peijnenburg 2013). The chemical structures of PFASs, for example PFOA and perfluorooctane sulphonate (PFOS) (Figs. 1 and 2), enable them to possess a combination of both hydrophobic properties from their perfluorinated structures, and hydrophilic properties from their functional groups ($-COO^-$ and $-SO_3^-$) (Fujii et al. 2007). These specific properties make PFASs to be used as surfactants, emulsifiers and performance chemicals.

Currently, PFASs are widely used in several applications, ranging from fire extinguishing foams, raincoats, non-stick cookware, medical implants, insecticides to military and aviation technology (Kärrman et al. 2007; German Federal Environmental Agency 2009; Whitworth et al. 2012). However, over the past few decades, these compounds have become a major health concern as they have been found in human tissues, blood, urine and breast milk (Harada et al. 2003; Hanssen et al. 2010; Lankova et al. 2013), with health impacts ranging from cancer, high cholesterol, obesity and allergies (Liu et al. 2010; Steenland et al. 2009; Stahl et al. 2011). In addition to being present in human tissues and fluids, prenatal exposure of foetuses has intensified concerns over the persistence of PFASs in the environment. Preliminary work has established that these compounds can cross the placenta from maternal blood, thus putting the foetus at risk, where exposure can lead to low birth weight and later life disorders such as increased risk to heart disease, developmental toxicity and Alzheimer's disease (Slotkin et al. 2008; Steenland et al. 2009; Hanssen et al. 2010; Whitworth et al. 2012). Moreover, the persistence of PFASs in breast milk has been documented, thereby increasing the risk of exposure even after childbirth (Gu et al. 2010).

The first recommended food for a new-born is normally the mother's milk as its components are important for the baby's nutritive requirements. Breast milk has cellular, immunochemical and biochemical constituents that can protect a new born from infections, diarrhoea and malnutrition, and is recommended from birth to at least 6 months (NICUS 2006). However, it has been acknowledged that lactation may be a significant pathway of PFAS exposure for neonates through breast milk, thus often used as a bio-indicator to estimate the body-load of some PFASs in infants (Rodriguez et al. 2009; Gu et al. 2010; Lankova et al. 2013). Additionally, based on body mass, infants are more vulnerable and are exposed to comparably elevated levels of chemicals than adults (Liu et al. 2011).

The two most studied and detected forms of PFASs are perfluorooctane sulphonate (PFOS) and perflurooctanoic acid (PFOA) which tend to have elimination half-lives of 5.4 years and 3.8 years respectively in human blood (Olsen et al. 2007). However, there is no documented information on their half-lives in human breast milk. A study by Haug et al. (2011) estimated that the total PFOA and PFOS in infants would

Table 1 Physicochemical properties of selected PFASs

Property	PFOS	PFOA	PFNA	PFHxS	PFDA	References
Molecular weight (g/mol)	500.13	414.07	464.08	400.12	514.09	Giesy et al. 2010; ATSDR 2015
Melting point (°C)	$\geq$ 400	54.3	62	274	79	ATSDR 2015; Haynes 2015; ChemicalBook 2012; ECHA 2015
Boiling point (°C)	249	192	218	239	218	Kosswig 2000; Haynes 2015; Savu 2000
Vapour pressure (mmHg) at 25 °C	2.9×10^{-3}	3.16×10^{-2}	8.3×10^{-2}	4.6×10^{-3}	1.01	Bhhatarai and Gramatica 2010; EPA 2012; Ding and Peijnenburg 2013
Solubility in water (mg/L) at 25 °C	3.2×10^{-3}	3.3×10^{3}	1.8×10^{-1}	6.2	2.8×10^{-2}	ATSDR 2015
logK_{ow} at 25 °C	4.49	4.81	5.48	3.16	2.90	EPA 2012; Ding and Peijnenburg 2013
pKa at 25 °C	0.14	1.33	−0.17	0.14	2.61	ATSDR 2015; Ding and Peijnenburg 2013
Bioaccumulation factor (L/kg)	3.2×10^{-1}	3.8×10^{-2}	3.98×10^{3}	5.01×10^{2}	7.94×10^{3}	Martin et al. 2003; Furdui et al. 2007; Houde et al. 2008

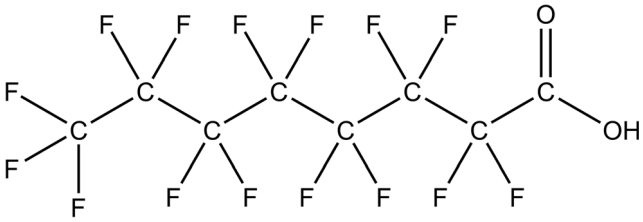

Fig. 1 Chemical structure of perfluorooctanoic acid (PFOA)

contribute > 83% and > 94% respectively, of exposure within 6 months through lactation. Such high exposure rates may in turn lead to numerous disorders later in life such as attention deficit hyperactivity disorder, high cholesterol and cardiovascular disease (Steenland et al. 2009; Ode et al. 2014). Some documented reports have associated the presence of PFOS in the human body to liver and bladder cancer, as well as contributing to reproductive and developmental toxicity during growth (Haddow et al. 1999; Thibodeaux et al. 2003; Inoue et al. 2004; Apelberg et al. 2007).

PFOA toxicity has been associated with accumulation in the blood, pancreas and liver (Olsen et al. 2007; ATSDR 2015) as well as damage to the thyroid and immune system, which may increase chances of allergies (Haddow et al. 1999). Nevertheless, conflicting results were reported by Okada et al. (2014), who concluded that there were no relationships between exposure to both PFOS and PFOA with allergies and infections in infants of 18 months. Research in rodents has also suggested that exposure to PFOA in toxic amounts leads to adverse developmental effects, which might also be exhibited in humans (Wolf et al. 2007; Rodriguez et al. 2009).

Another PFAS linked with detrimental health effects in infants is perfluorononanoic acid (PFNA). It is a characteristic persistent, non-volatile perfluoroalkyl found to target the liver, causing it to increase in weight (Wolf et al. 2010; Fang et al. 2008). In the USA, it has been reported that children between the ages of 12 and 15 years having elevated levels of PFNA, among other PFASs, were at a higher risk of attention deficit hyperactivity disorder (ADHD) (Hoffman et al. 2009). In a separate study, high PFNA levels indicated a positive correlation with increased cholesterol levels in USA adults aged between 20 to 80 years (Nelson et al. 2010). On the other hand, Steenland et al. (2009) mentioned that although high PFASs may lead to high cholesterol, which in turn predisposes children to higher risk of heart failure and obesity in the future, many of the epidemiological studies are cross-sectional and

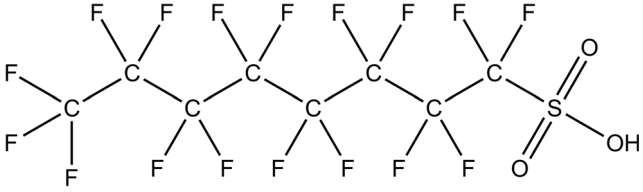

Fig. 2 Chemical structure of perfluorooctane sulphonate (PFOS)

therefore fail to clearly establish the underlying relationships between levels of exposure to PFASs and human health effects. Consequently, several regulatory and advisory bodies on environmental toxins, including the US Environmental Protection Agency's (US EPA) Science and Advisory Board, International Agency for Cancer Research (IACR) and US National Toxicology Program (NTP), have classified PFASs such as PFOA as potential carcinogens (Biegel et al. 2001; Lau et al. 2006; ATSDR 2015).

Numerous investigations have attempted to establish the main exposure pathway of PFAS in humans, but differing viewpoints make the results inconclusive (Harada et al. 2003; Fujii et al. 2007; Tittlemier et al. 2007; Scheringer et al. 2007; Hölzer et al. 2008; Stahl et al. 2009). While most studies have resolved that contaminated water and food play a key role, Ericson et al. (2008) investigated the relationship between exposure through contaminated food and acknowledged that a correlation between dietary intake and blood levels of PFASs did exist but however, concluded that diet was not the main route of exposure.

For infants, determining the concentrations of these organic compounds in the mother's milk may aid in estimating the level of exposure through their diet. To date, no experimental data exists that has established the routes of transfer of PFASs to breast milk from the mother's blood, where they are known to bind to serum proteins (Kärrman et al. 2010; Liu et al. 2010). The present review summarises the current data on PFASs in human breast milk, including the challenges of analysis as well as the possible modes of transfer from maternal blood to breast milk.

Structure of the review

Search strategies

Previously published data on the concentrations of PFASs in human breast milk from across the globe were summarised in this review. Suitable studies with reference lists published before 1 June 2017 were incorporated into the review. Literature searches were done using Scopus, PubMed, ScienceOpen, EuropePMC and SciFinder. Searched keywords included perfluorinated compounds, perfluorochemicals, breast milk, lactation, postnatal transfer, analysis and other associated terms. The titles and abstracts were first evaluated to determine those that qualified for a full-text review. The reference lists of the articles were also checked to find further literature.

Eligibility criteria

Articles that met the following criteria were eligible for the review: (1) those where the concentrations of PFASs in human breast milk were quantified, (2) those where the analytical method employed for the determination of PFASs was

specified and (3) where the participants were not occupationally exposed to PFASs.

Data extraction and assessment Data from the investigations that fell within the inclusion criteria were extracted and summated. PFAS concentrations were converted to pg/ml for ease of comparison and summarised into an evidence table (Table 2), which also included the following information: the region and country where the study was carried out, the number of participants, sample collection year and the first author and year of publication. Information obtained on the possible modes of transfer from maternal blood to breast milk was also summarised.

To assess the different analytical methods used to identify and quantify the concentrations of PFASs in breast milk, data such as the technique used for the analysis, the sample pretreatment method including extraction and cleanup methods, the mobile and stationary phases used, the percentage recovery obtained, and the first author and year of publication were extracted and summarised in Table 2.

Results and discussion

Evidence of PFASs in human milk

Despite the fact that PFASs have been synthesised for more than 60 years, their presence in human breast milk have only been studied in Asia, Europe and North America, with increased intensity over the past decade (Apelberg et al. 2007; Hoffman et al. 2009; Liu et al. 2010). The first published incidence of PFASs found in human breast milk was in 2004 (Kuklenyik et al. 2004), though other investigators later published PFAS concentrations from frozen breast milk samples collected as far back as 1996 and 1999 (Völkel et al. 2007; Tao et al. 2008a, b). Since then, several other studies have followed, thereby increasing concerns of exposure through lactation (Bernsmann and Fürst 2008; Kärrman et al. 2010; Liu et al. 2010; Lankova et al. 2013). It has been observed that the most xfrequently detected PFASs in humans include perfluorohexanesulphonic acid (PFHxS), PFOA and PFOS (Kärrman et al. 2007; Sündstrom et al. 2011).

Currently, while there are numerous reports of PFASs in the environment and in serum, no information is available for their occurrence in breast milk from Africa, Antarctica, Australia and South America (Table 2). Published data suggests a large variation in the geographical distribution of these compounds based on their structural profiles and their concentration levels. In North America, PFASs in breast milk have been quantified in Canada and the USA (Table 2) (Kubwabo et al. 2013; Kuklenyik et al. 2004; Tao et al. 2008a, b).

In America, high levels of perfluorohexanoic acid (PFHxA) and perfluoropeptanoic acid (PFPePA) in two samples were quantified to be 820 pg/ml and 1560 pg/ml respectively (Kuklenyik et al. 2004). It is difficult to explain the high levels of these two PFASs as there is no information available on the donors, sampling method or exact sampling location. Belgium, on the other hand, had one of the highest levels of PFASs recorded in literature, with peak levels of PFOS, PFOA and PFHxS being 28,200 pg/ml, 3500 pg/ml and 5300 pg/ml respectively (Roosens et al. 2010). In this study, the authors proposed that although more work was needed on the mode of transfer from maternal blood to human milk, the PFAS profiles and concentration levels suggested that sulphonates were easier to transfer to human milk compared to carboxylates (Roosens et al. 2010).

The highest concentrations of PFASs have been detected in breast milk samples collected from areas of high economic development and much industrial activity (Environmental Directorate OECD 2005; Tao et al. 2008a, b; Liu et al. 2010). For instance, in China, PFAS concentrations were quantified across 12 provinces, in both rural and urban areas. The results indicated elevated concentrations of PFOA (814 pg/ml), PFUnDA (196 pg/ml) and PFOS (100 pg/ml) in urban Shanghai where there is a high level of industrialisation. In contrast, the least concentrations were from rural Ningxia, one of the least developed areas in China, with concentrations values ranging from below the limit of detection (< LOD) for PFOA and PFUnDA to 6 pg/ml for PFOS (Liu et al. 2010).

Evidence from literature suggests a relationship between concentrations of PFASs and industrial development. Tao et al. (2008a, b) substantiated that individuals from countries with a high gross domestic product (GDP) had elevated concentrations of PFOS than those from countries with a low GDP. For instance, PFAS levels in samples from Cambodia, India and Vietnam were found to be 40–50% less than those reported in more developed countries such as China and Germany (Tao et al. 2008a, b). The levels of PFASs reported in samples from Asia showed that PFOS was the prime compound in human breast milk, with an estimated 85% occurrence in samples from India and 100% in other sampled countries (Tao et al. 2008a, b).

The highest PFOS concentrations ever recorded were from Ehime in Japan, with a mean of 232 pg/ml. These levels were attributed to the high usage and production of consumer goods containing these contaminants (Tao et al. 2008a, b). In more recent investigations, PFAS levels from other regions in Japan were at much lower concentrations than those from Ehime, although significantly still on the high end (Nakata et al. 2009; Fujii et al. 2012). These samples were collected from Hokkaido and Kyoto, with peak PFAS concentrations found for PFOA as high as 89 pg/ml and 93.5 pg/ml respectively.

Other high levels of PFOA in breast milk have been observed. In Germany, Suchenwirth et al. (2006) measured 12

Table 2 Reported data of frequently detected PFAS concentrations in human breast milk (pg/ml)

Europe

Country	Region	Sample number	Sample collection year	PFASs quantified above LOD	Mean (pg/ml)	Range (pg/ml)	Estimated daily intake (ng/kg body weight/day)	Reference
Belgium	11 provinces	22	2006	PFOS	–	< 400–28,200	–	Roosens et al. 2010
				PFOA	–	< 300–3500	–	
				PFHxS	–	< LOQ–5300	–	
Czech Republic	Olomouc	50	2010	PFOA	50	12–128	–	Lankova et al. 2013
				L-PFOS	33	7–114	–	
				Br-PFOS	20	< 10–63	–	
				PFNA	–	< 6–15	–	
				PFDA	–	< 6–12	–	
				PFHxS	–	< 6–22	–	
France	Not specified	30	2010	PFOS	78	24–171	–	Kadar et al. 2011
				PFOA	59	18–102	–	
Germany	Muttermilch	103	2006	PFOA	–	4100–12,700	–	Suchenwirth et al. 2006
Germany	North Rhine–Westphalian Sauerland	203	2006	PFOS	93	50–284	–	Bernsmann and Fürst 2008
				PFOA	176	80–610	–	
Germany	Munich	201	2007, 2009	PFOS	–	< 30–110	–	Fromme et al. 2010
				PFOA	–	< 150–250	–	
				PFHxS	–	< 20–30	–	
Germany	Munich	19	2006	PFOA	–	< 201–460	–	Völkel et al. 2007
				PFOS	116	28–239	–	
Germany	Leipzig	38	2006	PFOS	126	28–309	–	Völkel et al. 2007
Germany	Baviere	20	2010	PFOS	–	< 30–195	–	Mosch et al. 2010
				PFOA	–	> 150	–	
				PFHxS	–	> 20	–	
Hungary	Gyor	13	1996–1997	PFOS	317	96–639	–	Völkel et al. 2007
Italy	Bologna	37	2010	PFOA	60	24–241	–	Barbarossa et al. 2013
				PFOS	47	15–288		
Spain	Barcelona	20	2008	PFOA	–	< 15.2–907	–	Lorca et al., 2010
				PFOS	–	28–865	–	
				PFNA	–	< 11.5	–	
				PFDA	–	< 85.5–1095	–	
Spain	Valencia	10	2012	PFOA	177	12–980	94.8 ng kg − 1 per day (18.5 of PFOA, 12.2 of PFOS and 64.1 of the rest of PFASs)	Lorenzo et al. 2016
				ipPFNA	14	139–139		
				PFNA	4	2–21		
				PFOS	50	5–246		
				PFDA	43	1.4–306		
Spain	Murcia	67	2014	PFOA	54	< 10–211	7.15	Guzmán et al. 2016
				PFNA	41	15–70	–	
				PFDA	24	< 10–34	–	
Spain	Catalonia	10	2007	PFHxS	40	20–110	–	Kärrman et al. 2010
				PFOS	120	70–220	–	
Sweden	Uppsala	12	2004	PFHxS	85	31–172	–	Kärrman et al. 2007
				PFOS	201	60–470	–	
				PFOSA	13	< 7–30	–	
				PFOA	–	< 209–492	–	

Table 2 (continued)

Europe								
				PFNA	17	< 5–20	–	
Asia								
Cambodia	Phnom Penh	24	2000	PFOA	–	< 42.5–132	ΣPFOS, PFOA, PFHxS = 11.7	Tao et al. 2008b
				PFOS	–	17.2–327	ΣPFOS, PFOA, PFHxS, PFNA = 13.1	
				PFNA	–	< 8.82–12.3		
				PFHxS	–	< 1.66–18.6		
China	Beijing	30	2008–2009	PFOA	51.6	< 40–122	4.2	Fujii et al. 2012
				PFNA	15.3	< 10–47	1.3	
				PFDA	< 15	< 15–29	0.6	
China	Zhoushan	19	2004	PFOA	106.3	47–210	–	So et al. 2006
				PFOS	120.79	45–360	–	
				PFNA	18.1	6.3–62	–	
				PFDA	7.2	3.8–15	–	
				PFHxS	20.47	4.0–100	–	
China	12 Provinces	1237	2007	PFOA	116.0	< 14.15–814	88.4	Liu et al. 2010
				PFOS	16.2	6–137	1.4–15.9	
				PFNA	9.9	6–76		
				PFDA	-	< 1.44–63		
				PFHxS	37.6	< 0.69–15		
China	Jinhu	50	2009	PFOS	56	9–198	–	Liu et al. 2011
				PFOA	181	25–1440		
				PFNA	26	5–95		
				PFDA	20	< 1–70		
India	Chidambaram,Kolkata, Chennai	39	2002, 2004,2005	PFOA	–	< 42.5–335	ΣPFOS, PFOA, PFHxS = 10.1	Tao et al. 2008b
				PFOS	–	< 11.0–120	ΣPFOS, PFOA, PFHxS, PFNA = 11.0	
				PFNA	–	< 8.82		
				PFHxS	–	< 1.66–13.3		
Indonesia	Jakarta,Purwakarta	20	2001	PFOA	83.6	< 42.5	ΣPFOS, PFOA, PFHxS = 13.2	Tao et al. 2008b
				PFOS	–	25.4–256	ΣPFOS, PFOA, PFHxS, PFNA = 14.9	
				PFNA	–	< 8.82–135		
				PFHxS	–	< 1.66–6.23		
Japan	Kyoto	30	2010	PFOA	93.5	< 40–194	7.7	Fujii et al. 2012
				PFNA	32.1	< 10–72	2.6	
				PFDA	21.3	< 15–65	1.8	
Japan	Ehime	24	1999	PFOA	77.7	< 42.5–170	ΣPFOS, PFOA, PFHxS = 39.2	Tao et al. 2008b
				PFOS	232	140–523	ΣPFOS, PFOA, PFHxS, PFNA = 40.5	
				PFNA	–	< 8.82–23.9		
				PFHxS	7.55	< 1.66–18.2		
Japan	Hokkaido	51	Not Specified	PFOA	89	16–270	–	Nakata et al. 2009
				PFNA	35	8–124	–	
				PFOS	71	46–98	–	
				PFHxS	10	< 4–20	–	
Korea	Not Specified	17	2007	PFOA	41	< 43–77	4.7	Kim et al. 2011
				PFOS	61	32–130	0.8	
				PFHxS	7.2	0.83–16	6.9	
Korea	Seoul	30	2010	PFOA	64.5	< 40–173	5.3	Fujii et al. 2012
				PFNA	14.7	< 10–41	1.2	

Table 2 (continued)

Europe								
				PFDA	< 15	< 15–19	0.6	
Korea	Group 1; $n = 215$ (Seoul, Chungcheong, Honam, Youngnam) Group 2; $n = 50$ (Suwon, Wonju, Daegu,Gwangju, Jeju)	264	2013	PFOA PFOS	71 49	52–110 31–77	5.0–11.0 3.4–7.5	Kang et al. 2016
Malaysia	Penang	13	2003	PFOA	–	< 42.5–90.4	ΣPFOS, PFOA, PFHxS = 19.7	Tao et al. 2008b
				PFOS	121	48.7–350	ΣPFOS, PFOA, PFHxS, PFNA = 20.9	
				PFNA	–	< 8.82–14.9		
				PFHxS	6.45	< 1.66–13.3		
Philippines	Quezon	24	2000, 2004	PFOA	–	< 42.5–183	ΣPFOS, PFOA, PFHxS = 19.8	Tao et al. 2008b
				PFOS	97.7	27.0–208	ΣPFOS, PFOA, PFHxS, PFNA = 21.2	
				PFNA	–	< 8.82–25.0		
				PFHxS	15.8	< 1.66–58.9		
Vietnam	Hanoi, Ho Chi Minh	40	2000, 2001	PFOA	75.8	< 42.5–89.2	ΣPFOS, PFOA, PFHxS = 13.1	Tao et al. 2008b
				PFOS	6.81	16.9–393	ΣPFOS, PFOA, PFHxS, PFBS, PFHpA, PFNA = 14.1	
				PFNA		< 8.82–10.9		
				PFBS		< 1.11–3.98		
				PFHxS		< 1.66–26.8		
				PFHpA		< 4.45–6.88		
North America								
Canada	Ontario	13	2003–2004	PFOA	178	–	–	Kubwabo et al. 2013
				PFNA	34	–	–	
				PFDA	0.4	–	–	
				PFHxS	26	–	–	
				PFOS	26	–	–	
USA	Information of location not available	2	2003	PFHxA	820	–	–	Kuklenyik et al. 2004
USA	Massachusetts	45	2004	PFOA	43.8	< 30.1–161	1.7	Tao et al. 2008a
				PFNA	7.26	< 5.2–18.4	–	
				PFOS	131	< 32.0–617	14.7	
				PFHxS	14.5	< 12.0–63.8	–	
				PFDA	–	< 7.72–11.1	–	

pooled samples from 103 participants and presented the highest levels of breast milk PFOA in literature, which ranged from 4100 to 12,700 pg/ml. These findings were even higher than levels reported in the North Rhine–Westphalian Sauerland region, with known PFAS pollution from industrial wastes and contamination by a recycling company (Bernsmann and Fürst 2008). The results from the pooled samples were unusually distributed and distinctly higher than their matching PFOS levels and thus, should be interpreted with care. No explanation was given concerning these high levels. In the USA, a decrease in the levels of PFASs has been reported in both humans and the environment as a result of the voluntary phasing out of the manufacturing of PFOS bulk material in 2001 by the 3 M Company, a known leading producer of PFOS (Kannan et al. 2004; Olsen et al. 2007). However, PFOA levels have not declined as anticipated, with records showing that PFOA serum levels remained constant between April 2003 and August 2007 and may apparently be increasing (Olsen et al. 2008; Beesoon et al. 2011).

Nursing history and PFAS concentration

A study by Tao et al. 2008b showed that previous breast feeding may lower the concentrations of PFOA in breast milk. Corresponding to this, numerous studies have found that mothers who were nursing for the first time had a higher load of PFASs compared to those who had breast fed before (Thomsen et al. 2010; Kadar et al. 2011; Barbarossa et al. 2013; Guerranti et al. 2013; Guzmán et al. 2016).

Thomsen et al. (2010) showed that the levels of PFASs in breast milk of non-occupationally exposed multiparous women who breast fed were comparatively lower than those who were breast feeding for the very first time (Thomsen et al. 2010). The mean concentrations of PFOS and PFOA were less by 44% and 59% respectively in women who had previously nursed compared to those who were nursing for the first time. In a similar study in Italy, PFOS levels were quantified above the LOD in 90% of the breast milk samples from primiparas women, while they were quantified in 62% of breast milk from multiparous mothers (Barbarossa et al. 2013). In these samples, the concentration levels of PFASs in primiparas mothers ranged from 15 to 288 pg/ml (mean 57 pg/ml) and from 15 to 116 pg/ml (mean 36 pg/ml) in multiparous women.

In Spain, the overall concentrations of PFASs ranged from 13 to 397 pg/ml (mean 96 $\pm$ 101 pg/ml) in women who were nursing for the first time, while in those who had previously nursed, the values ranged from 13 to 167 pg/ml (mean 40 pg/ml $\pm$ 31 pg/ml) (Guzmán et al. 2016). In addition, PFOA levels above the LOD were detected in all the samples from primiparas participants, while only 42% of the samples from multiparous donors contained PFOA. The literature data above, including other studies (Hamm et al. 2010; Mondal et al. 2014; Kishi et al. 2017), suggest that breast milk acts as the prime exposure pathway of environmental PFASs for breast fed infants whilst acting as a route for progressive elimination of these compounds from the mother's body.

Noting the significant relationship that may exist between concentrations of PFASs and the number of previous pregnancies, it is possible to propose that first-born children would likely experience higher exposure dose of PFASs compared to subsequent siblings. However, this would only be applicable if the exposure of the mother remains the same over a stated period. Should exposure of the mother increase (body burden) after the first birth, there is a likelihood of corresponding increase in the birth that would follow. Whitworth et al. (2012) stated that the intervals between pregnancy also had a significant influence on the body burden of PFASs, suggesting that should there be a prolonged gap between births, contamination of breast milk may end up being as high as that of the first lactation. To date, this hypothesis has neither been opposed nor confirmed in the scientific community.

A number of preliminary studies have indicated that PFAS concentrations are often gender specific in non-occupationally exposed populations, where females generally have lower concentrations compared to men (Olsen et al. 2003; Calafat et al. 2006; Kärrman et al. 2007). Depuration of these chemicals through long-term breast feeding could be one of the ways responsible for this observation.

Possible mode of transfer from maternal blood to breast milk

PFASs have been found to persist in various organs and other matrices of the human body, including the liver, spleen, urine and serum (Lau et al. 2007; Olsen and Zobel 2007; Fromme et al. 2010). Levels of PFASs in breast milk have been reported to be lower than PFAS levels in maternal plasma and serum in matched samples collected from the same donors (Hinderliter et al. 2005; Kärrman et al. 2007; Liu et al. 2010). In an animal study, the concentrations of PFOA in rats' plasma were reported to be ten times higher than those in the matched milk samples (Hinderliter et al. 2005). On the other hand, PFOS concentrations in human breast milk were found to be 1.6% and 0.5% of the levels in blood from Spanish nursing mothers (Kärrman et al. 2010) and Chinese mothers (So et al. 2006), respectively. Although matching blood and milk samples from the same donors has been an attempt to establish the transfer efficiency and pathway of different PFASs from maternal blood to breast milk, the mechanism of transfer has not been clearly documented.

While the mode of transfer of PFASs is still unclear and very debatable, no experimental data is currently available to provide insights on the systemic circulation of PFASs in humans. Furthermore, recent publications have also noted that the profiles of some PFASs in breast milk differ from those in blood samples from the same donors (Kim et al. 2011; Fujii

et al. 2012), thus adding more ambiguity to their transfer mechanism. However, many researchers have agreed that once in the body, PFASs do not accumulate in lipids but rather in protein-rich organs such as the kidneys, blood and liver (Martin et al. 2004; Kannan et al. 2004; Völkel et al. 2007; MacManus-Spencer et al. 2010). In blood, these contaminants have been noted to form strong bonds with the protein albumin, accounting for their persistence in the serum (Jones et al. 2003; Butenhoff et al. 2006; Liu et al. 2011).

Serum albumin is considered the most abundant protein in human blood as its concentrations range from 35 to 50 gL^{-1} (Peters Jr 1985), making it the preferable mode of transport to distribute biological components, such as hormones, as well as PFASs to various organs and tissues in the body (Jones et al. 2003; MacManus-Spencer et al. 2010). A study by Han et al. (2003) has reported that albumin in the serum has a substantial binding capacity for PFOA, where more than 90% of the compound in blood binds to the protein. This study further demonstrated that albumin has 6–9 binding sites per molecule available for PFOA, thereby leaving an estimated < 5% of the compound as a free fraction in the plasma (Han et al. 2003). In a separate study on the interactions between PFOS and proteins, Jones et al. (2003) noted that PFOS had the potential to displace lipids and steroid hormones from the binding sites on serum proteins, thus causing the contaminants to be classified as an endocrine disruptor. This study concluded that PFOS has a high propensity for albumin, making it unavailable for interaction with other proteins or molecules until all the accessible binding sites on serum albumin have been occupied (Jones et al. 2003).

Andersen et al. (2008) indicated that the modes of action for PFASs are largely determined by both their chemical and physical properties. In this regard, these compounds simulate fatty acids in both their structure and chemistry and are thus most likely transferred to the mammary gland by binding to albumin the same way fatty acids do (Peters Jr 1985; MacManus-Spencer et al. 2010). The structural similarity of PFASs to fatty acids suggests the possibility of PFASs to pass across the mammary epithelial membrane the same way fatty acids do. McManaman (2014) outlined the different processes involved in milk production, as well as the transport of fatty acids needed for milk lipid synthesis. The outline indicated that natural long-chain fatty acids in humans are first hydrolysed by lipoprotein lipase and then transported to the mammary gland by serum triglycerides or carrier proteins, including albumin. Once at the external plasma membrane, they are then transferred through regulation of fatty acid transporters in mammary membranes. These transporters include CD36, acyl-coA synthetase (ACSL), fatty acid translocase (FAT) and fatty acid transport proteins (FATP) (McManaman 2014). An example of structural similarities between PFASs and naturally occurring fatty acids is PFOA (Fig. 1) as previously described, and caprylic acid (octanoic acid) (Fig. 3), an eight-carbon saturated fatty acid found naturally in the fat component of milk from many mammals (Beare-Rogers et al. 2001).

The credit of the protein-binding mechanism is that it explains why PFAS concentrations in milk are lower than those in serum. However, the limitation of this protein-binding mechanism is that the high binding efficiency of PFASs to serum protein limits the possibility of these contaminants to be passed through to breast milk and ideally should not be persistent in the same. However, the high levels found in milk samples in some preliminary data such as 820 pg/ml of PFHxA in USA (Kuklenyik et al. 2004) and 317 pg/ml of PFOS in Belgium (Völkel et al. 2007) propose that PFASs do accumulate in high amounts in breast milk. As aforementioned, once PFASs are bound to albumin in serum, there is very limited interaction of these compounds with other molecules, and therefore would suggest further limited possibility of their interaction with fatty acid transporters in mammary membranes. This then suggests that the method of transfer should therefore allow for large amounts to pass to breast milk without being restricted by binding to serum albumin. In this regard, a membrane-transport mechanism could be responsible for the transfer of unbound PFASs from maternal blood to breast milk (Figs. 4 and 5).

Transport of chemicals into human breast milk involves the passage of low molecular weight chemicals through the mammary epithelium membrane which is semi-permeable (Black 1996). While smaller molecules can easily pass through this membrane through diffusion, larger compounds can be transferred into the alveolar cells by the process of pinocytosis, where particles or liquids are engulfed and released back into breast milk through apocrine secretion (Casey 2005). This could account for the prevalence of short-chain PFASs in breast milk compared to long-chain PFASs (Andersen et al. 2008; Kim et al. 2011; Fujii et al. 2012; Kang et al. 2016). Short-chain carbons are more soluble and have a lower molecular weight, which makes them more readily available to pass through the mammary epithelial membrane and thus contaminate breast milk. This suggests that chain length plays a key role in the transfer of these contaminants from the vascular system to breast milk and influences the pharmacokinetic properties of PFASs, where smaller molecules are eliminated faster, even via depuration (Ohmori et al. 2003). In addition, Fujii et al. (2012) showed that among

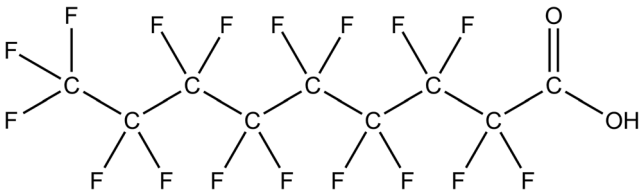

Fig. 3 Chemical structure of perfluorononanoic acid (PFNA)

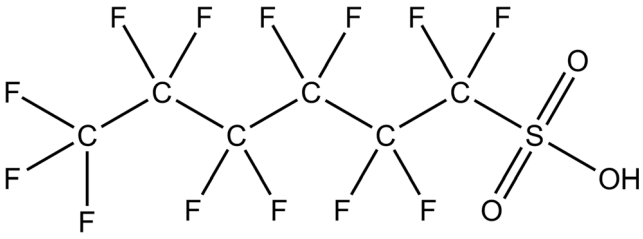

Fig. 4 Chemical structure of perfluorohexane sulphonic acid (PFHxS)

the long-chain compounds (C_{10}–C_{13}) found in breast milk of mothers in three Asian countries, odd numbered PFASs were more common than even numbered PFASs, apart from PFDA in Japanese samples. The reason for this observation is still unclear.

The challenge with the membrane-transport mechanism is that PFASs are generally lipophobic and do not accumulate in lipids, thus they are likely not to be transported through serum lipids across the mammary membrane as other chemicals do. However, the fact that PFASs are similar to fatty acids, suggests a strong possibility that they can be transported to breast milk through the same mechanism.

A conclusive preference mode of transfer between these two mechanisms cannot be established based on the current data. However, it can be opined that both mechanisms may work together, to varying degree, to ensure transfer of PFASs from the maternal vascular system to breast milk.

Analytical challenges for the determination of PFASs in breast milk

The complexity in the physical and chemical properties of PFASs presents various challenges during analysis, particularly for complex biological matrices such as breast milk. The analytical procedures for PFASs usually follow the general strategies for the analysis of complex organic compounds in biological matrices, with a common extraction and cleanup steps followed by chromatography and MS detection. In this review, we emphasised on methods focussing on breast milk as a matrix. Table 3 presents different methods reported in the literature for the analysis of PFASs covering the period 2004 to 2017.

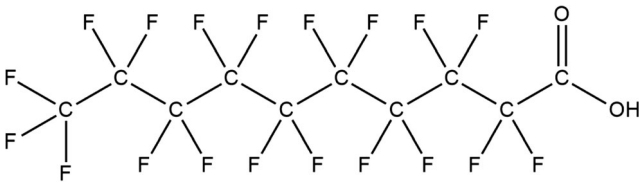

Fig. 5 Chemical structure of perfluorodecanoic acid (PFDA)

Sample collection and storage

Contamination can occur during collection and storage of breast milk samples for analysis, particularly where collection bottles have not been pre-treated or the wrong bottle material is used (Martin et al. 2004; Okáľová 2014). PFASs have been noted to attach to container surfaces due to their ionic properties and this has been a major challenge for both sampling and storage, such that they are unavailable for analysis at trace levels (Reagen et al. 2008). Glass containers have been deliberated as suitable options for collection, but it has also been argued that ionic PFASs similarly adsorb onto the glass surfaces (Martin et al. 2004; Van Leeuwen and de Boer 2007).

The use of polypropylene containers for the collection and storage of breast milk for PFAS analysis is very popular in the scientific literature. This is due to the fact that polypropylene containers have lower affinity for surface adsorption compared to other materials (Bernsmann and Fürst 2008; Fromme et al. 2010; Barbarossa et al. 2013). Prior to use, sample collection containers are usually rinsed with polar or semi-polar solvents such as methyl tert-butyl ether, methanol and acetone to reduce contaminations (van Leeuwen and de Boer 2007).

Sample extraction and cleanup

The complexity of the sample treatment is related to potential matrix interference and the separation technique used, usually tandem LC MS (Trojanowicz et al. 2011). Similarly, the physiochemistry of the analyte, mostly the polarity of PFASs, have to be considered. Recent advances in extraction methods together with the parallel improvement of the techniques of analysis has resulted in the reduction in the complexity of the sample pre-treatment and has increased the accuracy and precision of the analysis of PFASs. The matrix in which specific compounds to be measured exist need to be considered for suitable sample preparation and cleanup. Human breast milk is a combination made up of different constituents which vary depending on the maturity of the milk. Colostrum is the first milk produced immediately after birth, and its composition differs from that of mature breast milk in that its protein content is higher and the carbohydrate content lower. The fat content remains consistent during lactation but slightly increases with each nursing (Jennes 1979). Many researchers use mature milk samples to measure the levels of PFASs present.

The composition of breastmilk usually makes it a very complex matrix for the analysis of trace contaminants. The binding of PFASs to proteins, particularly albumins, has meant that numerous investigators employ protein precipitation using organic solvents such as methanol and acetonitrile or by freeze-drying for sample pre-treatment (Fromme et al. 2010; Mosch et al. 2010; Thomsen et al. 2010). In their

Table 3 Analytical methods reported in the literature for the determination of PFASs in breast milk

Technique	Sample pre-treatment	Extraction/cleanup (cartridge used)	Mobile phase	Stationary phase	Amount of breast milk sample used	Analyte	Average percentage recovery	Reference
HPLC-TIS-MS/MS	Formic acid added, vortex mixed and sonicated	SPE (60 mg/3 mL Oasis HLB column)	A: 20 mM ammonium acetate (pH 4) in water B: 20 mM ammonium acetate (pH 4) in methanol (flow rate of 300 μL/min)	Betasil C8 column (3 × 50 mm, 5 μm)	1 ml	PFOSA PFHxS PFOS PFPeA PFHxA PFOA PFNA PFDeA PFUA PFDoA	82 82 74 80 102 89 80 62 55 26	Kuklenyik et al. 2004
HPLC-ESI-MS/MS	Freeze-drying	SPE (Oasis HLB) (adapted from Kuklenyik et al. 2004 with modifications)	A: 2 mM ammonium acetate B: Methanol (flow rate of 300 μL/min)	Keystone Betasil C18 column (2.1 × 50 m, 5 μm)	2 ml	PFBS PFHxS PFOS PFHxA PFHpA PFOA PFNA PFDA PFUnDA	86 92 93 88 92 92 92 92 94	So et al. 2006
HPLC-ES- MS/MS	–	SPE (Waters Oasis WAX) (Adapted from Taniyasu et al. 2005)	A: 2 mM ammonium acetate in methanol B: 2 mM ammonium acetate in water (flow rate of 300 μL/min)	Discovery HS C18 Column (50 mm × 2.1 mm inner diameter, 3 μm, 120 Å pore size)	1 ml	PFBuS PFHxS PFOS THPFOS PFDS PFHxA PFHpA PFOA PFNA PFDA PFUnDA PFDoDA PFOSA	79 81 83 51 72 80 84 82 77 43 38 39 34	Kärrman et al. 2007
LC-ESI-MS/MS	Hydrolysis of protein and fat using Protease Type XIV (Sigma L 1754-5G) and Lipase Type VII (Sigma P 5147-1G)	SPE (Strata X SW-SPE column) (adapted from Karrman et al. 2004)	A: 2 mM ammonium acetate in water B: Methanol (flow rate of 150 μl/min)	C18 Column (Agilent, 100 × 2.1 mm, 1.8 μm)	3 ml	PFBS PFPA PFHxA PFHpA PFHxS PFHpS PFOA	Recovery at 1.25 μ-g/l 92.1 80.1 93.0	Bernsmann and Fürst 2008

Table 3 (continued)

Technique	Sample pre-treatment	Extraction/cleanup (cartridge used)	Mobile phase	Stationary phase	Amount of breast milk sample used	Analyte	Average percentage recovery	Reference
						PFNA PFOS PFDA PFDS PFUnA PFDoA	88.1 103.6 93.0 95.4 98.5 88.2 94.9 57.7 65.8 95.6	
HPLC-ESI-MS/MS	Internal standards added and samples sonicated with 90.2% formic acid	SPE (Waters Oasis WAX; 6 cc,150 mg) (adapted from Kuklenyik et al. 2004, with modifications)	A: 2 mM ammonium acetate B: Methanol (flow rate of 300 μL/min)	Betasil® C18 column (100 × 2.1 mm, 5 μm)	5 ml	–		Tao et al. 2008a
HPLC-ESI-MS/MS	Internal standards added and samples sonicated with 90.2% formic acid	SPE (Waters Oasis WAX; 6 cc,150 mg) (adapted from Tao et al. 2008a)	A: 2 mM ammonium acetate B: Methanol (flow rate of 300 μL/min)	Betasil® C18 column (100 × 2.1 mm, 5 μm)	5 ml	–		Tao et al. 2008b
HPLC-ESI-MS/MS (Adapted from Brink et al., 2006)	Internal standards added and samples sonicated with 600 μl acetonitrile	–	100% of a 2 mM ammonium acetate buffer solution (flow rate of 0.6 ml/min)	Reprosil-Pur C18 AQ, Dr. Maisch, Ammerbuch, Germany (5 μm, 33 mm × 3 mm)	400 μl	–		Völkel et al. 2007
LC-MS/MS	–	Ultrasonic extraction followed by SPE (Oasis WAX)	20 μmol/L Ammonium acetate in water (flow rate of 0.2 mL/min)	Betasil® C18 (2.1 × 50 mm, 5 μm)	–	PFHxS PFOS PFOA PFNA	101 105 109 94	Nakata et al. 2009
LC-TIS-MS/MS	Hydrolysis of protein and fat using Lipase Type VII (Enzyme Commission number: 3.1.1.3; EC: 232–619-9) and Protease Type XIV (mix of different enzymes; EC: 232–909-5)	On-line SPE (Oasis HLB 20 × 2.2, 25 μm) (adapted from Mosch et al. 2010)	A: 2 mM ammonium acetate B: Methanol	Reprosil-Pur C18 AQ, Dr. Maisch, Ammerbuch, Germany (5 μm, 33 mm × 3 mm)	0.4 ml	–		Fromme et al. 2010
UPLC-ES-MS/MS	–	SPE			1 ml	–		

Table 3 (continued)

Technique	Sample pre-treatment	Extraction/cleanup (cartridge used)	Mobile phase	Stationary phase	Amount of breast milk sample used	Analyte	Average percentage recovery	Reference
		(Waters Oasis WAX)	A: 2 mM ammonium acetate in methanol B:2 mM ammoniu m acetate in water (flow rate of 400 μL/m)	Waters Acquity UPLC® BEH C18 Column (2.1 × 50 mm, 1.7 μm)				Kärrman et al. 2010
UPLC-ES-MS	Internal standards added and samples sonicated with formic acid. Final solution filtered. (adapted from Liu et al. 2008)	SPE (Waters Oasis WAX 60 mg/3 mL)	A: 2 mM ammonium acetate B: Methanol (flow rate of 0.4 mL/min)	Waters Acquity UPLC® BEH C18 Column (2.1 × 50 mm; 1.7 μm)	2 ml	PFHxS PFHpS PFOS PFPeA PFHxA PFHpA PFOA PFNA PFDA PFUdA	Recovery at 100 pg/-mL 114 110 96 136 99 95 110 98 102 57	Liu et al. 2010
LC – ESI-MS/MS	Enzymatic protein hydrolysis and protein precipitation	On-line SPE (Oasis HLB 20 × 2.2, 25 μm)	A: 2 mM ammonium acetate B: Methanol	Reprosil-Pur C18 AQ, Dr. Maisch, Ammerbuch, Germany (5 μm, 33 mm × 3 mm)	0.4 ml	73–112% of all PFASs		Mosch et al. 2010
UPLC-ES-MS	Internal standards added and samples sonicated with formic acid. Final solution filtered. (adapted from Liu et al. 2008)	SPE (Waters Oasis WAX) (adapted from Liu et al. 2008)	A: 2 mM aqueous ammonium acetate solution B: Methanol (flow rate of 0.4 mL/min)	Waters BEH C18 Column (2.1 × 50 mm; 1.7 μm)	2 ml	PFHxS PFOS PFDS PFPeA PFHxA PFHpA PFOA PFNA PFDA PFUdA PFDoA PFTrDA	Recovery at 0.1 ng/-mL 83 94 49 112 87 119 93 97 89	Liu et al. 2011

Table 3 (continued)

Technique	Sample pre-treatment	Extraction/cleanup (cartridge used)	Mobile phase	Stationary phase	Amount of breast milk sample used	Analyte	Average percentage recovery	Reference
							89 75 36	
UPLC-ESI-MS/MS -	Formic acid and water added to samples	SPE (Waters Oasis WAX) (adapted from Taniyasu et al. 2005)	Ammonium hydroxide in acetonitrile	ACQUITY BEH C18 column (2.1 × 50 mm; 1.7 mm, Waters, USA)	1 ml	Ranged between 78% and 101% for PFOS and PFOA. For PFNA, PFHxA, PFBS and PFHxS averages recoveries varied between 81% and 109%. Recoveries of PFBA and PFDA were 70% and 160%, respectively.		Roosens et al. 2010
LC-ESI-HRMS	–	LLE followed by a purification on two successive SPE (Oasis HLB and carbon graphitized Envicarb cartridges)	A: Methanol B: 20 mM Ammonium acetate (flow rate of 0.6 mL/min)	Gemini C18 reverse phase Column (3 µm, 50 × 2.0 mm)	3 ml	PFBS PFHxS PFHpS PFOS PFBA PFPeA PFHxA PFHpA PFOA PFNA PFDA PFUnA PFDoA PFOSi	Recovery at 750 pg/-mL 79 83 104 89 82 61 69 78 87 78 82 81 102 82	Kadar et al. 2011
HPLC-ESI-MS/MS	Protein precipitation and hydrolysis using proteas and lipase	IPE (adapted from Taniyasu et al. 2005) Followed by SPE (adapted from Tao et al. 2008a) (Waters WAX; 6 cc, 150 mg)		C18 column				Kim et al. 2011
GC-EI-MS	Freeze-drying			J &W DB-5MS column	2 ml	–		

Table 3 (continued)

Technique	Sample pre-treatment	Extraction/cleanup (cartridge used)	Mobile phase	Stationary phase	Amount of breast milk sample used	Analyte	Average percentage recovery	Reference
		LLE followed by SPE (Presep-C silica gel column)	Helium carrier gas (flow rate of 1.5 mL/min)					Fujii et al. 2012
UPLC-ESI-MS/MS	Protein precipitation with acetone	LLE followed by a purification on two successive SPE systems (Oasis HLB cartridge then Supelclean™ ENVI-Carb cartridge)	A: Ammonium acetate 20 mM aqueous solution B: Methanol (flow rate of 0.5 mL/min)	Waters Acquity UPLC® BEH C18 reversed phase column (50 × 2.1 mm, 1.7 μm)	3 ml	–		Barbarossa et al. 2013
LC–ESI-MS/MS	–	SPE (HLB glass cartridges)	A: 20% acetonitrile in 2 mM ammonium acetate B: 90% acetonitrile in 2 mM ammonium acetate (flow rate of 200 μl/min)	Two Discovery HS C18 columns (7.5 cm 2.1 mm, 3 μm)	1 ml			Kubwabo et al. 2013
UHPLC-ESI-MS/MS	Formic acid and acetonitrile added, hand mixed	d-SPE (C18)	A: 5 mM ammonium acetate B: Methanol (flow rate of 0.3 mL/min)	Waters Acquity UPLC HSS T3 analytical column (100 mm × 2.1 mm, 1.8 μm)	15 ml	PFBA PFPeA PFHxA PFHpA PFOA PFNA PFDA PFUdA PFDoA PFTrDA PFTeDA PFBS PFHxS	92 91 97 95 104 94 97 99 90 101 98 99 101	Lankova et al. 2013
GC-EI-MS (Adapted from Dufková et al. 2009, 2012)	–	IPE (adapted from Guerranti et al. 2013)	Helium carrier gas (flow rate of 1 mL/min)	J &W DB-35MS column (30 m × 0.25 mm × 0.25 μm)	0.5 ml	Percentage mean recovery for all PFASs was 96.71%		Guzmán et al. 2016

Table 3 (continued)

Technique	Sample pre-treatment	Extraction/cleanup (cartridge used)	Mobile phase	Stationary phase	Amount of breast milk sample used	Analyte	Average percentage recovery	Reference
HPLC-ESI-MS/MS	Protein precipitation and hydrolysis using proteas and lipase	IPE followed by SPE (adapted from Kim et al. 2011 with modifications)	A: 2 mM aqueous ammonium acetate solution B: Methanol (flow rate of 200 μL/min)	YMC-Pack ODS-AQ C18 column, (2.0 × 150 mm, 3.0 μm)	–	Ranged between 80% and 120%, except for PFPeA (72.5–86.1%)		Kang et al. 2016
UHPLC-ESI-MS/MS	Alkaline digestion (adapted from Lorca et al. 2010)	SPE (Strata-X 33 m polymeric reversed phase 60 mg)	Ammonium hydroxide in methanol	Kinetex 1.7 V XB − C18 column (50 × 2.1 mm and 5 μm)		PFBA PFPeA PFBS PFHxA PFHpA PFHxS PFOA PFHpS ipPFNA PFNA PFOS ipPFNS PFDA PFDS PFDoDA PFTrDA PFTeDA PFHxDA PFODA PFUnDA	Recovery at 75 ng L^{-1} 90 81 91 81 90 98 96 79 89 80 112 107 90 91 91 89 97 98 92 108	Lorenzo et al. 2016

methodology, Bernsmann and Fürst (2008) applied a hydrolysis step before protein precipitation, utilising a protease and a lipase enzyme to break down the milk emulsion and discharge the PFASs bound in the micelles to improve detection and quantification. The results showing the success of the hydrolysis were a change in the pH of the samples from 7.5 to 6.9, and a clearly visible breakdown of the emulsion after incubation (Bernsmann and Fürst 2008).

Different extraction protocols have been reported for PFASs in breast milk. However, solid phase extraction (SPE) is more popular owing to high recovery, short analysis times, simpler procedures and use of less solvents compared to other techniques such as liquid/liquid extraction (LLE). Although SPE is a refined alternative to LLE, several authors employed a combination of LLE and SPE for sample extraction and cleanup, since LLE is efficient in the extraction of compounds with high molecular weights (Kadar et al. 2011; Fujii et al. 2012; Barbarossa et al. 2013), this is followed by SPE cleanup of the extracts (Table 3). LLE on its own is not effective as an extraction method since some PFASs do not partition in the presence of LLE organic solvents, which then results in low recoveries (Jones-Lepp et al. 2004). Moreover, SPE is preferred as both extraction and cleanup can be performed simultaneously.

SPE cartridges that have been commonly used for PFASs are hydrophile-lipophile balance cartridges (HLB), carbon-18 (C_{18}) cartridges, silicon cartridges and weak anion exchange cartridges (WAX) (Hu and Yu 2010; Sun et al. 2017). In some investigations involving milk as the matrix, reversed phase SPE cartridges packed with silica have been used to further clean up extracts and to also reduce interfering compounds (Hu and Yu 2010; Fujii et al. 2012; Lorenzo et al. 2016). Results in these studies have reported lower matrix effects. WAX cartridges, however, have been distinguished as providing the best extraction outcomes during SPE for both the perfluoroalkyl carboxylic acids and the perfluoroalkyl sulphonates as they have been noted to reduce matrix effects (Sun et al. 2017). HLB cartridges, on the other hand, provide good extraction recovery only for the long-chain PFASs. While C_{18} cartridges have been found to contain trace amounts of PFASs and are less recommended (Yamashita et al. 2004; Wang et al. 2014; Sun et al. 2017), their ability to provide high recovery and suitable peak shapes for certain PFASs such as PFOS and PFOA have prevented their phase-out (Schiessel and Krepich 2017). In addition, C_{18} phases normally require an adjustment of pH to 2 by adding formic acid so as to increase its capacity to trap the required analytes.

On-line SPE have been employed for extensive biomonitoring which requires large throughput and high sensitivity (Mosch et al. 2010; Schiessel and Krepich 2017). This extraction technique has been used in milk samples as well as several other aqueous matrices and it has shown several advantages over the conventional SPE methods. Firstly, the conventional SPE method requires the methanol extract to be evaporated before reconstitution, whereas on-line SPE bypasses this stage and therefore takes less time to complete (Schiessel and Krepich 2017). Furthermore, on-line SPE requires a smaller volume of the sample to be used (Table 3) and offers more sensitivity due to the lower injection volume.

Recent studies have also used ion-pairing extraction (IPE) either in conjunction with SPE or as the sole extraction method (Kim et al. 2011; Guzmán et al. 2016; Kang et al. 2016). This method is mainly used because of the acidic nature of PFASs, where an ion-pairing agent is added to the sample to improve the extraction efficiency of the analytes (Villaverde-de-Sáa et al. 2012). Nevertheless, this process is often followed by successive SPE cleanups, which makes sample preparation tedious and not cost-effective.

Instrumental analysis

Gas chromatography Methods based on gas chromatography (GC) coupled with mass spectrometer (MS) as the detector have been used for the identification and quantification of PFASs in various environmental matrices (Trojanowicz et al. 2011). Although GC was earlier used to study the levels of PFASs in both indoor and outdoor air, it has also been used in breast milk analysis as it offers high separation efficiencies for individual PFASs (Table 3) (Shoeib et al. 2006; Fujii et al. 2012; Trojanowicz and Koc 2013; Guzmán et al. 2016). In addition, the good peak resolution offered, GC helps to determine branched isomers and their distribution in a sample (Trojanowicz and Koc 2013). However, they have since become very unpopular due to tedious derivatisation steps occasioned by the general low volatility of PFASs.

In environmental samples, GC is often used when the target analytes are PFASs or fluorotelomer alcohols which are more volatile than the acids. Guzmán et al. (2016), however, quantified both PFAAs and PFASs in breast milk using GC-MS by first esterifying PFOA, PFNA, PFDA, perfluoroundecanoic acid and perfluorododecanoic acid using chloroformates to make them semi-volatile by forming methyl esters. During sample preparation for GC analysis, derivatisation can be done using diazomethane; benzyl bromide during LLE; 2,4-difluoroaniline; strong anion exchange extraction in conjunction with methyl iodide; or using methanol or butanol (Martin et al. 2003; Orata et al. 2009; Trojanowicz and Koc 2013). However, the major challenge with the derivatisation of PFASs is the formation of unstable derivatives, which makes it difficult to obtain reproducible data with GC. In addition, GC methods involve long, multiple sample preparation stages before analysis, which in turn subject the whole process to multiple sources of human error (Larsen and Kaiser 2007) and loss of analytes. Although the use of GC with electron capture detector (ECD) has been proposed for the analysis of PFASs, the strong bonds between the fluorine and carbon

atoms in the chemical structures of PFASs, renders ECD with low specificity (Okáľová 2014).

Liquid chromatography methods The primary analytical technique used for the determination of PFASs in breast milk is liquid chromatography (LC) with various detectors such as mass spectrometer (MS) tandem electrospray ionisation mass spectrometry (ESI-MS/MS) and high-resolution mass spectrometer (HRMS) (Table 3). LC methods have been more popular than GC methods owing to their good selectivity and high sensitivity for a wider range of PFASs (Yusa et al. 2012). In recent time, ultra-high-performance liquid chromatography (UPLC-MS/MS) is becoming very popular in the analysis of PFASs due to the use of ultra-small particle size (such as 1.7 μm) stationary phases and very high pressure offered by UPLC results in shorter analysis time and better LOD compared to HPLC-MS (Trojanowicz and Koc 2013). As shown in Table 3, various columns have been employed for the separation of PFASs; however, as highlighted above, C18 and HBL shorter columns with small particle size are becoming more popular (So et al. 2006; Kärrman et al. 2007; Nakata et al. 2009; Lorenzo et al. 2016). The reduced internal diameter reduces separation speed and increases analysis time, thereby giving better peak resolution of each PFAS at trace level. The use of reverse phase chromatography applying standard mobile phases such as acetonitrile, methanol and water offers great selectivity, sensitivity and good peak quality, hence its popularity in the analysis of PFASs. To improve the ionisation efficiencies of PFASs in negative ESI-MS/MS mode, most authors have included ammonium acetate buffer to mobile phases (Haug et al. 2009).

Electrospray ionisation in the negative polarity mode has been the ionisation technique of choice when analysing PFASs in breast milk by numerous investigators (Tao et al. 2008a, b; Kärrman et al. 2010; Roosens et al. 2010; Kim et al. 2011). The advantage of ESI is that it is significantly sensitive and offers high selectivity in the measurement of PFASs and has been esteemed as the first reliable method for analysing PFASs (Reagen et al. 2008). As PFASs are often required to be detected at trace levels, ESI becomes the most suitable ionisation method since it offers low sensitivity usually in the range 2 to 100 $pgml^{-1}$ in biological samples (Okáľová 2014; Sun et al. 2017). Furthermore, Table 3 indicates the popularity of small internal diameter columns among investigators.

A major challenge of ESI, however, is matrix effect induced by either signal suppression or enhancement (Yusa et al. 2012). Signal enhancement occurs when matrix components or interferences, particularly lipids from the breast milk, reduce surface tension during detection and thereby make the ionisation detection signals stronger (Enke 2007; Sun et al. 2017). Signal suppression, on the other hand, occurs when co-eluting residual components compete with the target analytes for charge and in turn reduce the number of analyte ions available, thus resulting in erroneous detection (Enke 2007). To overcome matrix effect in ESI, most authors have introduced the use of matrix-matched calibrations and carbon-13 labelled stable isotopes or deuterated analogues as internal standards for the quantitation of PFASs in biological matrices such as breastmilk (Villagrasa et al. 2006; van Leeuwen et al. 2008; Sun et al. 2017). Similarly, in order to overcome the shortcomings of ESI, liquid chromatography high resolution mass spectrometry (LC-HRMS) has been applied for the analysis of PFASs in human milk samples from France (Kadar et al. 2011). The main strength of this system is that the high-resolution technique reported minimal matrix effects hence reducing the risk of either underestimating or overestimating the levels of PFASs in the samples. Since most PFASs do not contain any chromophore, analysis of PFASs with HPLC coupled with ultraviolet-visible spectroscopy (UV/Vis) detectors is limited.

Quality control

There is an overall struggle for quality with regard to PFASs in human breast milk samples as there are no standard reference materials or stable isotope internal standards specifically for milk samples. The various methods that have been published, with both their strengths and limitations, are not standardised and many of these are only validated in-house, hence posing a loop-hole in data accuracy. Because many of these methods were initially dedicated to monitoring PFASS in food, water and blood, they demonstrate some limitations with regard to their specificity and sensitivity to breast milk. Therefore, there is need for a validated reference method, which defines the accuracy, stability, recovery, calibration, precision, sensitivity and uncertainties of the method.

Conclusion

Intake of breast milk by infants is a major exposure source of perfluoroalkyl substances through depuration by the mother. Notable effects of such post-natal exposure have been recorded in literature and these vary from developmental, neurological and reproductive effects. On the other hand, it has also been noted that breastfeeding acts as an elimination pathway from the mother as investigations have found that previous breastfeeding tends to reduce the levels of PFASs in breastmilk. Although there is strong evidence of the persistence of these contaminants in human milk, many of the published material is based on small sample size and thus should be interpreted with care. Large gaps still exist in literature of their geographical profiles and distribution as there are no recorded documents of these compounds from Africa, South America and Australia.

The mode of transfer from maternal blood to breast milk has not yet been scientifically established, thus we proposed that transfer could be through both protein binding and membrane transport mechanisms. Although a conclusive preferred mode of transfer between these two cannot be established based on current data, it can be submitted that both mechanisms may work together to varying degree. Understanding the mode of transfer could be the key to limiting transfer of these and other organic pollutants to infants through breast feeding. In addition, a lot of attention in the studies of PFASs is more focused on long chain compounds, yet preliminary studies indicate that short-chain compounds are predominant in breast milk. Research on the differences in levels of PFASs in colostrum and matured milk could also be beneficial in identifying the most vulnerable breast-feeding stage for infant exposure. In addition, it has been suggested that depuration of these compounds through breast feeding could be one of the ways responsible for females having lower levels of PFASs than men; further studies may need to be done to elaborate on this as menstruation and delivery are also possible excretion methods.

Generally, there have been great advancements in the development of various analytical methods to quantify PFASs in breast milk, but a standard validated method specific to breast milk is lacking within the scientific community which is key, as reliable data are crucial in understanding the toxicity and the fate of these contaminants. Many challenges in analysis have been noted when it comes to PFASs, mostly related to their physicochemical properties. Firstly, these properties limit the types of methods that can be used to quantify them, for instance, they do not have chromophores therefore ultraviolet-visible spectroscopy cannot be used. They also have low volatility and form unstable derivatives, which in turn limits the use of GC-MS. One other major challenge of analysis is that they form strong anions which enable them to bind to laboratory equipment, containers and solvents, making them unavailable for analysis at trace levels.

In conclusion, there remain gaps in literature where more work focussing on the fate, transfer mechanism and accurate analytical procedure for the determination of PFASs in breast milk is needed to further understand the fate, load and exposure mechanisms of PFASs. Generally, literature data suggests a global decline in PFAS levels in breast milk, therefore the benefits of breast feeding may outweigh the potential risks associated thereof.

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